BD Directigen Flu A+B

See package insert for more information

Specimen Handling:
Transport fresh specimens to the lab as rapidly as possible in a suitable liquid transport system maintained on ice or refrigerated at 2-8°C. Process specimens as soon as possible after collection. If necessary, specimens may be stored at 2-8°C for up to 72 hours or at -20°C for up to 7 days from time of collection. Fresh specimens are preferable to frozen, as decreased sensitivity may result. It is essential that correct specimen collection and preparation methods be followed. Specimens obtained early in the course of illness will contain the highest virus titers.

Specimen Preparation:
Acceptable specimens include nasopharyngeal washes, nasopharyngeal aspirates, nasopharyngeal swabs, lower nasal (turbinate) swabs, and bronchoalveolar lavages.

Procedure for use with Nasopharyngeal Washes and Bronchoalveolar Lavages:
1. Wash or lavage volumes of 2-3mL are recommended.
2. Excessive wash or lavage volumes may result in decreased test sensitivity.
3. Process specimen as described in "Test Procedure".

Procedure for use with Nasopharyngeal Aspirates:
1. Nasopharyngeal aspirate specimens of less than 0.5mL in volume must be dispersed in 2mL of transport medium or saline prior to processing.
2. Aspirate specimens of greater than 0.5mL require a transport medium or saline volume addition of greater than or equal to 4mL.
3. Process specimen as described in "Test Procedure".

Procedure for use with Nasopharyngeal, Lower Nasal, or Throat Swabs:
1. Throat swab specimens may be processed without dilution in transport medium by direct extraction as described in "Test Procedure." Throat swabs may also be added to 1-2mL of transport media or saline immediately after collection.
2. Nasopharyngeal and lower nasal swab specimens should be placed into 1-2mL of transport medium or saline immediately after collection.
3. Mix the swab well in the transport medium or saline solution.
4. Remove as much liquid from the swab as possible.
5. Discard the swab into appropriate container.
6. Process specimen as described in "Test Procedure".

Test Procedure:
Place a **DispensTube** in the designated area of the workstation.

A. **ColorPAC Device Preparation**
   Remove a **ColorPAC** device from its foil pouch immediately before use.
   Push down to ensure Flow Controller is seated snugly in both wells in the ControlPAC device.
B. **Specimen Extraction**

-All samples except throat swabs without transport media: Gently mix **Reagent E**, and dispense 8 drops into a **DispensTube**. Mix specimen well. Pipette 200µL of specimen into the **DispensTube**.

-Throat swabs without transport media: Gently mix **Reagent E**, and dispense 16 drops into the **DispenTube**. Swirl swab while intermittently squeezing swab through the walls of the **DispensTube** for 15-30 seconds. Remove swab while squeezing to displace excess liquid.

***Quality Control:** Dispense 8 drops of **Reagent E** into the **DispenTube**, followed by 4 drops of well-mixed **Control A+/B-** or **Control B+/A-.** Mix well. Follow Test Procedure to dispense extracted **Control A+/B-** in an alternating dropwise manner into both wells of a single test device. The **Control A+/B-** serves as the positive for control Flu A as well as the negative control for Flu B. Repeat procedure in a separate device with **Control B+/A-**. The **Control B+/A-** serves as the positive control for Flu B as well as the negative control for Flu A.

- Insert a **DispensTube** tip into the **DispensTube**. Vortex or mix thoroughly.
- Invert the **DispensTube and holding the tube on the upper half, away from the tip,** gently squeeze. **Squeezing the tube close to the tip may result in ejection of the tip and leakage of contents from the tube.**
- **Dispense the extracted specimen dropwise (avoiding excess bubble addition), alternating single drops between the A and B test wells until 4 drops have been added to each well of the ColorPAC test device. Thus a total of 8 drops from each extracted specimen is added to a single test device.**
- **When testing extracted Controls, the extracted Control A+/B- must be added to both wells of a single ColorPAC test device, and similarly, the extracted Control B+/A- must be added to both wells of a single ColorPAC test device.**
- Allow specimen to absorb completely.
- If specimen fails to be absorbed into the device within 5 minutes, dilute as described in "Specimen Preparation" section and retest.

C. **Color Development:**

- **Remove the Flow Controller. Discard as biohazard.**
- **Reagent 1** – gently mix. **Add 2 drops to each well.** Allow to absorb completely.
- **Reagent 2** – gently mix. **Add 2 drops to the A well only.** Proceed immediately to Reagent 3 addition.
- **Reagent 3** – gently mix. **Add 2 drops to the B well only.** Allow Reagents in A and B wells to absorb completely. **Allow to stand 2 minutes.**
- **Reagent 4** – gently mix. **Add 3 drops to each well.** Allow to absorb completely.
- **Reagent 5** – gently mix. **Add 3 drops to each well.** Allow to absorb completely.
- **Reagent 6** – gently mix. **Add 3 drops to each well.** Allow to absorb completely. **Allow to stand 5 minutes.**
- **Reagent 7** – gently mix. **Add 2 drops to each well.** Allow to absorb completely.

Interpretation of Results:

A positive result should be reported as positive for the presence of influenza A and/or B antigen. A negative result should be reported as a presumptive negative for the presence of influenza A/B antigen.

Positive Test for Flu A – A purple triangle appears in the A well on the ColorPAC membrane and indicates influenza A antigen was detectable in the specimen. The background area should be a light yellow to light purple color. A purple control dot should be evident in the center of the triangle unless obscured by an intense positive reaction.

Positive Test for Flu B – A purple triangle appears in the B well on the ColorPAC membrane and indicates influenza B antigen was detectable in the specimen. The background area should be a light yellow to light purple color. A purple control dot should be evident in the center of the triangle unless obscured by an intense positive reaction.

Negative Test for Flu A or Flu B – No purple triangle is visible in either the A well, or the B well, or both wells, indicating that influenza A antigen, or influenza B antigen, or both were not detectable in the specimen. A purple color dot appears in either the A well, the B well, or in both wells on the ColorPAC membrane indicating proper performance of test procedures and reagents. The background area should be a light yellow to light purple color.

Uninterpretable Test – The test is uninterpretable either for Flu A or Flu B, or for both, if neither purple dot nor purple triangle is visible in the respective wells. Any incomplete triangle is also to be regarded as an uninterpretable test. If uninterpretable, the test hould be repeated.

The test is also uninterpretable either for Flu A, or for Flu B, or both, if a white triangle appears on the ColorPAC membrane **and** the entire surrounding background membrane is purple in color. A muted control dot may be evident in the center of a white triangle. Additionally, the test result is uninterpretable if the entire membrane area is purple and no control dot is observed. To correct these problems, dilute the sample either 1:4 in 0.9% saline or in transport media and repeat the test.

Excessively mucoidal samples may fail to be absorbed through the ColorPAC membrane or may yield uninterpretable results. These specimens may be diluted 1:4 with saline, mixed well, and retested.